LARVAL TREMATODES FROM AUSTRALIAN FRESHWATER MOLLUSCS PART XIV

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Cercaria natans n. sp.
(Fig. 1-6)

In December 1948 a new echinostome cercaria was found infecting 2 of 77 *Planorbis isingi*. The infected snails were collected from a small shallow lagoon beside the River Murray at Wood's Flat near Blanchetown. In January 1949, 2 out of a total of 236 snails were found infected in the same locality. In February 1949 the same cercaria was found again, this time at Tailem Bend, where one snail from a total of 28 was infected. Although *Planorbis* snails were collected from the swamp at Wood's Flat in April 1949 and at Tailem Bend in the following June, no infections with this parasite were found.

Under laboratory conditions the cercariae were observed to emerge from the host snail at about mid-day, and after about two hours of swimming they encysted in the host snail. On some occasions one cercaria only was given off during a day. They swam about or floated in the bottom of the tube with periodic excursions towards the surface of the water. When at rest on the bottom or suspended in the water the body was curved and made an obtuse angle with the tail.

Measurements were made after fixing by the addition of an equal volume of hot 10% formalin to the quantity of water in which the cercariae were swimming. In fixed specimens the body is flexed. Measurements based on 20 such specimens are:—body length, 252-269μ; breadth, 184-176μ.

Average length of 10 living specimens in fairly extended condition is 460μ. Diameter of acetabulum, 100μ; of oral sucker, 66μ; giving a sucker ratio of 5:3. The acetabulum has a fringed margin. The tail, 460-482μ in length in fixed material, is longer than the body, and, like it, is capable of a considerable degree of extension. There is a dorsal as well as a ventral fin fold on the distal half of the tail, but these folds do not extend to the tip. They can be seen best when a little pressure is exerted on the cercaria after staining with dilute neutral red. There are fin folds at the base of the tail also, but they appear to have no connection with those situated distally (fig. 2, 3). The tip of the tail which is free of any fin fold, is capable of contraction and extension, as well as threshing movements, quite independently of the rest of the tail.

The collar of 35 spines is not readily visible in living specimens but is obvious in killed material. There are 5 corner spines on each side ventrally, about 5 lateral spines on each side in a single row, and the rest are arranged in two alternating rows dorsally. The spines widen slightly at about the middle of their length and then taper to a broad point. The corner spines are larger than the rest. The dorsal alternating spines of the two series are all the same size (fig. 5). Average measurements which were made from the metacercaria, are:—corner spines, 15·5μ long; lateral, 14μ; dorsal, 13·5μ. Spinules cover both dorsal and ventral surfaces of the cercaria; they are most abundant anteriorly and ventrally.

There is a prepharynx followed by a spherical pharynx. The oesophagus, when seen from the ventral aspect, appears to be composed of a single column of about 8 crescentic cells. It bifurcates just anterior to the acetabulum and the caeca extend almost to the posterior end of the body.

The details of the excretory system are difficult to determine. A long descending tube on each side opens into a terminal bladder which at times has

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the shape shown in fig. 1. This tube is widest at the level of the acetabulum and is packed with granules from this point to near the anterior loop where the granules become smaller.

The ascending tube is narrower than the descending and runs parallel with it. At a point half-way between the acetabulum and the front of the bladder it divides into an anterior and a posterior collecting tubule. The anterior one extends to the level of the pharynx where a group of 3 flame cells is connected

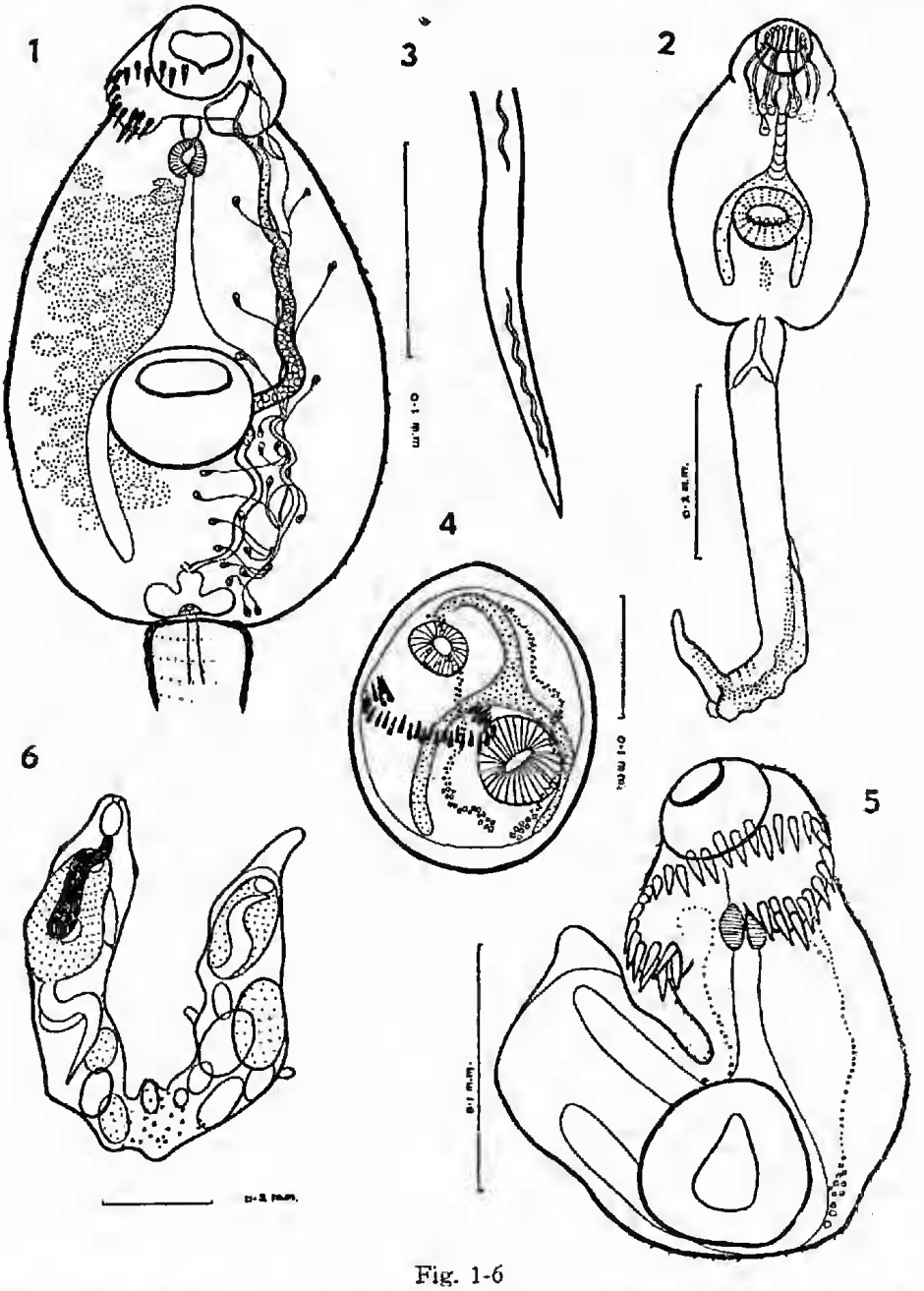

Fig. 1-6

Cercaria natans—1, cercaria showing collar spines and excretory system; 2, cercaria showing gland cells and fin folds after staining with neutral red; 3, sketch of dorsal view of tail, indicating anterior and posterior fin folds; 4, cyst; 5, metacercaria; 6, redia.

with it. Between this point and the acetabulum there are two pairs of flame cells. Level with the acetabulum are two groups each of 3 flame cells, and at the point where the ascending tube divides there is another group of probably 3, though only 2 flame cells of this group were actually observed. Posterior to this point there are three groups, each of 3 flame cells, giving a total of 25 on each side. Ciliary flames are present in the descending and ascending tubes. The arrangement of the groups of flame cells is shown in fig. 1. A caudal excretory pore opens dorsally at the base of the tail. From the bladder an excretory duct extends into the tail, and at a point about 70μ from the base of the latter, divides into two.

On the dorsal lip of the mouth are the openings of eight ducts which can be traced backwards to about the level of the pharynx, on each side of which are about 4 pyriform gland cells, which stain only very faintly with neutral red. On either side of the oral sucker is a group of greenish refractive bodies. Granular cystogenous cells are densely aggregated beneath the cuticle from about the level of the pharynx to the posterior end of the body (fig. 1).

Rediae containing living cercariae were dissected from the liver of the snail. They contained up to five or six cercariae. Nearly all the rediae possessed bright orange pigment spots. Anteriorly the pharynx opens into a short darkly coloured intestine which occupies only about one-eighth of the body length. The collar is not obvious but in some specimens a birth pore can be seen opening a short distance from the anterior end. Foot processes are short and of equal length and are more obvious in young specimens (fig. 6).

We successfully infected the pond snail, *Amerianna* sp., and the tadpole of *Limnodynastes tasmaniensis* with the cercaria. The host snail, *Planorbis isingi*, is a host also for the metacercaria, the cysts occurring mainly in the liver among the rediae. Generally the host snail bears a large number of cysts. Measurements of 30 cysts from *Planorbis* ranged from 176μ x 176μ to 191μ x 206μ. In the experimentally infected *Amerianna* sp. 10 cysts were found in each snail, mainly in the tissue of the mantle, and measurements of 20 of these cysts ranged from 168μ x 183μ to 191μ x 191μ. Two cysts were dissected from one of the infected tadpoles (fig. 4) and only one cyst from the other. They were found in the peritoneum surrounding the kidney and in the kidney tissue. Measurements of two of these cysts were 229μ x 168μ and 168μ x 139μ.

The cysts are thick-walled and difficult to break but metacercariae could be expressed from them in some cases. The spines of the metacercaria are larger than those of the cercaria and their arrangement is shown in fig. 5. Their measurements have been given above. The spination of the body of the metacercaria is more pronounced than in the cercaria. The digestive system is similar and the sucker ratios are the same. The acetabulum lies in the posterior half of the body.

This cercaria belongs to the *Echinostomum* group as the spines of the collar are in a double row, uninterrupted dorsally, and the spines of the two dorsal rows are equal in size.

The presence of a fin membrane on the tail, according to Sewell (1922) separated his *Cercaria Indica XLVIII* from other Echinostomes. He placed it with others in a specially erected group—the Echinatoides group. Our cercaria in some respects falls into this group. However, the presence of spines on the body and the fact that the excretory tubule divides into anterior and posterior tubules at a point half-way beween the acetabulum and the bladder would, according to Sewell's diagnosis, exclude our cercaria from that group.

Wesenberg-Lund (1934) placed *C. echinostomi* Dubois in the Echinatoides group and stated that at first he determined this species as *C. limbifera* Seifert 1926 (later described by Brown 1931), but that after seeing Brown's description he decided that the two species were distinct though closely allied. Like our species the two cercariae just mentioned have 35 spines but both are larger; *C. limbifera* has hairs anteriorly; and both *C. limbifera* and *C. echinostomi* have fin folds extending to the tip of the tail.

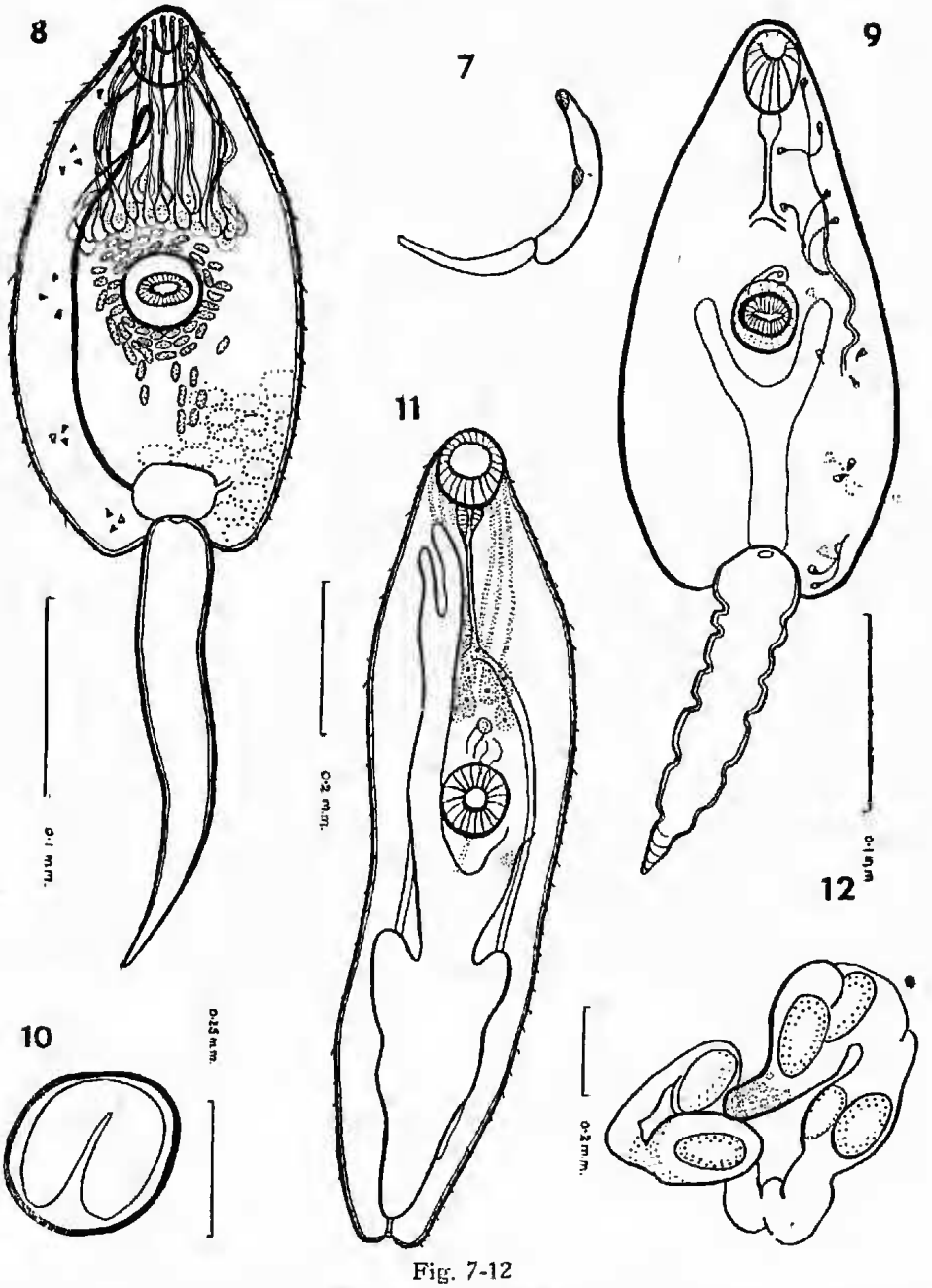

Fig. 7-12

Cercaria lethargica—7, sketch, showing usual flexed condition; 8, ventral view, showing gland cells, also pigmented cells surrounding acetabulum; 9, excretory system; 10, cyst; 11, metacercaria; 12, sporocyst.

We might mention having found in *Amerianna pyramidata* and *Planorbis isingi* from Wood's Flat in February 1949 an echinostome cercaria possessing an anatomy very like that of *C. natans* but having 37 spines. It is not the larva of *Echinostomum revolutum* because it has dorsal and ventral fin folds like *C. natans*. Its gland cells stain very readily with dilute neutral red whereas those of *C. natans* are very difficult to stain even after prolonged immersion in the dye. Its metacercaria has been obtained in the host snails, *Amerianna pyramidata* and *Planorbis isingi*, and in the tadpole of *Limnodynastes tasmaniensis*.

Cercaria lethargica n. sp.

(Fig. 7-12)

Since December 1937 a gymnocephalous cercaria has been found infecting the gastropod, *Plotiopsis tatei*, at various places along the lower River Murray. In April 1939, at Tailem Bend, it was found in 40 out of 200 of these molluscs. After February 1940 the incidence of the infection decreased until in February 1948, out of 250 *Plotiopsis* collected near Mannum, only one snail was found infected with this cercaria. In April 1949 at Wood's Flat, near Blanchetown, 1 out of 30 snails was found infected. The parasite has never been collected earlier than December but has been found as late as June. It is not an actively moving cercaria but remains for long periods suspended in the water with the tail, which is attached ventrally, and the body forming a continuous curve so that the whole organism is crescent-shaped (fig. 7). When in movement the tail is a little longer than the body and in this extended condition the cuticle is smooth. In a contracted state, as in preserved specimens, the cuticle is thrown into folds which become smaller towards the tip of the tail.

Measurements of the body of 30 specimens after fixation by the method described above, ranged from 268μ in length by 114μ in breadth to 190μ in length by 130μ in breadth. The breadth was measured across the widest part of the cercaria, just posterior to the acetabulum. The oral sucker and the acetabulum are approximately equal, with an average diameter of 32μ. The tail varies in length from 153μ to 268μ. The cuticle of the cercaria bears small spines which are arranged in rows and are embedded for part of their length in the cuticle. They are more obvious anteriorly than posteriorly.

Around the acetabulum and to a less extent behind it, the cercaria is coloured a yellowish-brown due to the presence of many cells containing yellowish granules. Beneath these cells are densely granular cystogenous cells which stain readily with many dyes. These latter cells do not extend much further forward than the acetabulum.

The oral sucker is oval in some preserved and extended specimens, round in living material. The mouth is subterminal. The pharynx can be seen only in stained preparations. The oesophagus is even more difficult to observe but in one or two stained specimens it could be followed to its bifurcation about midway between the oral and ventral suckers. Caeca were not observed.

Anterior to the ventral sucker and extending forwards to a point about midway between the suckers is a conspicuous group of clear gland cells from which ducts extend forwards to open on the oral sucker in 3 groups, a dorsal and two ventro-lateral.

The bladder in its contracted state is approximately circular. Extended, it forms a conspicuous Y with a broad stem and with the arms reaching forwards on each side of the acetabulum. An excretory pore opens into the depression into which the tail fits. When the bladder is contracted an excretory duct may be seen extending from it on each side to the level of the pharynx where it forms

a loop (fig. 8). When the bladder is in its extended condition, excretory ducts can be seen anteriorly but their points of entry into the bladder could not be detected. Several groups of flame cells have been seen (fig. 9). They are in groups of 3, two groups are anterior to the acetabulum and there are perhaps 3 groups behind it.

A rudimentary cirrus sac lies partly anterior to and partly dorsal to the acetabulum and terminates in the midline or slightly to one side, at the genital pore.

The liver of the host snail may contain many sporocysts varying in length from 1·5 to 2 mm. At intervals along their length, between the contained cercariae, they are constricted and have the appearance of a string of beads. There are many refractive globules which in some specimens collect at one end of the sporocyst (fig. 12).

Successful experimental infections were carried out using the aquarium fish, *Gambusia*. In 1940 when many infected snails were present with the fish, as many as 56 cysts were found in the muscles and body cavity of one fish, and 3 others in the liver. In the experimental infections carried out in 1948 cysts were found in the liver only, in the two fish which were successfully infected. These cysts measured from 311μ x 311μ to 328μ x 303μ.

The metacercaria, when excysted from the thick-walled cyst (fig. 10), shows many of the characters of the cercaria. The body has lengthened and measures from 1·1 mm. - 1·2 mm. in length and from 250μ - 229μ in breadth. The oral and ventral suckers which are about equal in size, measuring 78μ - 82μ, retain their positions relative to each other. The spination is more marked than in the cercaria.

The digestive system is observable in stained specimens. The oesophagus bifurcates about midway between the suckers and the caeca extend to the posterior end. A group of gland cells is present as in the cercaria, and their ducts open on the oral sucker. A few yellowish granular cells which were a feature of the cercaria, are present near the acetabulum.

The most obvious feature of the metacercaria is the excretory system which still retains its Y-shape. However, the arms of the Y extend anteriorly until they are level with the pharynx and the outline of the Y is not as well defined as in the cercaria. The excretory system is crowded with refractive material which makes the metacercaria appear dark and obscures other features of the living specimen. When the metacercaria is fixed and stained other structures can be seen (fig. 11).

There is a genital pore and cirrus sac in about the midline some distance anteriorly to the ventral sucker. A fine tube (possibly the uterine rudiment) extends from behind the ventral sucker to the region of the cirrus sac. Posterolaterally from the acetabulum is a group of cells which may be the ovarian rudiment.

This cercaria belongs to the Leptocercous group of Lühe (1909). Sewell (1922) modified Lühe's scheme of classification and included a number of different groups in the Gymnocephalous cercariae. That classification was modified by Dubois (1929) and Wesenberg-Lund (1934). Sewell (1922) has described a cercaria very like ours—*C. indica XIV*. He stated that it fell into no known group or sub-group. It differs from our species however in size, amount of pigment, and in the arrangement of its excretory canals and tubules, although it has the Y-shaped bladder. The excretory system of our cercaria is more like that of an echinostome and it is therefore possible that the adult may belong to the family Psilostomatidae in which the life history is similar to that of Echinostomes. However, the Psilostome cercariae described by Beaver (1939)

and by Szidat (1937) are different in many respects from ours and their development occurs in rediae not in sporocysts. The Y-shaped excretory system, packed with refractive material, of the metacercaria of our species is suggestive of the Fellodistomatidae.

Summary

A new 35-spined echinostome cercaria, *C. natans,* is described from *Planorbis isingi* from the lower Murray. The metacercaria has been obtained experimentally from the gastropods, *Planorbis isingi* and *Amerianna* sp., and from the tadpole of *Limnodynastes tasmaniensis.*

A closely allied 37-spined cercaria is reported from *Amerianna pyramidata* and *Planorbis isingi,* its metacercaria having been obtained experimentally from these two species of snails as well as from the tadpole just named.

Cercaria lethargica n. sp. is described from the gastropod, *Plotiopsis tatei,* the metacercaria occurring (experimentally) in a fish, *Gambusia.* The adult is perhaps a Psilostome or a Fellodistome.

Acknowledgments

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